



N₂O and NO dynamics in AOB-enriched and mixed-culture biomass: Experimental Observations and Model Calibration

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Abstract

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Nitrous oxide (N2O) is emitted during biological nitrogen removal (BNR) in wastewater treatment operations. The main organisms responsible for N2O production are ammonia-oxidizing bacteria (AOB) and heterotrophic denitrifying bacteria (HB). AOB produce N2O (1) during incomplete ammonium (NH4+) oxidation to nitrite (NO2-) (nitrifier nitrification, NN) and (2) under low dissolved oxygen (DO) conditions using NO2- as the terminal electron acceptor (nitrifier denitrification, ND). In heterotrophic denitrification N2O is an obligate intermediate of respiration that can be released under low carbon-to-nitrogen ratios or in the presence of DO (HD).

Mechanistic models can be useful to synthesize the complex interrelationships within BNR to ultimately develop mitigation strategies for N2O emissions. In this study a combination of experimental and modelling tools were used to study and describe N2O dynamics from N-removing processes.

N2O production dynamics were investigated using targeted batch respirometric assays with two biomass types: an AOB-enriched biomass (type A) and a mixed liquor with a lower AOB abundance from a full-scale BNR plant (type B). Nitrogenous substrates (NH4+, NO2-, NH2OH) were added at varying oxygen concentrations while responses in N2O, nitric oxide (NO), dissolved oxygen and pH were monitored.

Under aerobic conditions net N2O production with biomass type A and B was higher during NH2OH oxidation compared to NH4+; no N2O was produced in the sole presence of NO2-. At the onset of anoxia the N2O production significantly increased, accumulating in the bulk. Biomass type B showed a much higher N2O consumption rate compared to type A, explained by a larger fraction of N2O reducers. NO production in both systems was triggered by NH2OH pulses under any DO level and NO2- pulses at low DO concentrations.

A newly developed pseudo-mechanistic model distinguishing N2O production pathways from autotrophic (NN, ND) and heterotrophic bacteria (HD) successfully described the experimental data.

The model considers NH3 as substrate of AOB and could describe NH4+ oxidation at varying NH4+ and pH. NH2OH and NO - intermediates of AOB and HB metabolism - were the key precursors of N2O production. The model captured a pH dependence of the N2O consumption rate of biomass type B (pH optimum = 8).

Parameter sets were estimated for each biomass type (maximum rates, substrate affinities) and highlighted differences in microbial community composition. For example, the estimated NH3 affinity differed, probably due to the different NH4+ and pH levels at which the biomasses operated ($\text{NH}_4^+_{\text{type A}} \rightarrow \text{NH}_4^+_{\text{type B}} \rightarrow K_{\text{NH}_3_{\text{type A}}} > K_{\text{NH}_3_{\text{type B}}}$). The fractions of NH4+ oxidized and NO2- reduced to N2O by AOB (NN and ND pathways) also varied between systems. The model could also describe the sequential accumulation of heterotrophic denitrification intermediates in biomass type B from NO3- to N2O via NO2- and NO. Overall, while biomass type B showed a larger N2O production rate, the net emission remained lower as heterotrophic denitrifiers acted as an N2O sink.